Experimental and Toxicologic Pathology 67 (2015) 237–243

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Histopathologic changes in liver and kidney tissues induced by carbaryl in *Bufotes variabilis* (Anura: Bufonidae)

Özlem Çakıcı*

Ege University, Science Faculty, Biology Department, Zoology Section, Bornova, İzmir, Turkey

ARTICLE INFO

Article history:
Received 2 July 2014
Received in revised form 20 October 2014
Accepted 9 December 2014

Keywords:
Carbaryl
Bufotes variabilis
Toad
Liver
Kidney
Histopathology

ABSTRACT

The purpose of this work was to investigate for the first time histopathologic effects of carbaryl in liver and kidney tissues of *Bufotes variabilis*. After 96 h following exposure to carbaryl (low dose: 0.05, medium dose: 0.1 and high dose: 0.2 mg/g), the toads were euthanized and dissected. In liver tissue, vacuolization in hepatocytes, necrosis, mononuclear cell infiltration, an increase in melanomacrophage number, enlargement of sinusoids, hemorrhage and congestion were determined in exposed toads. In kidney tissue, mononuclear cell infiltration, hypertrophied Bowman's capsule cells, deformation, vacuolization, karyolysis and necrosis of renal tubule epithelium, brush border destruction, glomerular shrinkage, hemorrhage and fibrosis were observed in carbaryl-treated groups. According to this investigation, carbaryl caused histopathologic damages in liver and kidney tissues of *B. variabilis*.

1. Introduction

Because of their wide range of use, pesticides exist as common contaminants in soil, air, water and on non-target organism. Therefore, they can harm many species including useful soil microorganisms and insects, non-target plants, fish, birds and other wildlife (Aktar et al., 2009). Today, it is a known fact that many amphibian species show substantial declines in number and distribution due to this high rate usage of pesticides (Davidson, 2004). According to Garber et al. (2007), carbaryl especially has adverse effects on anuran populations. Carbaryl (1-Naphthyl-N-methylcarbamate) is a broad spectrum pesticide and widely used as a contact and systemic insecticide on agricultural products (Murty et al., 2012). N-methylcarbamate insecticides cause cholinesterase inhibition. This lead to overstimulation of the cholinergic system (McDaniel et al., 2007). By means of acetylcholinesterase, the neurotransmitter acetylcholine (ACh) is broken down to choline and acetic acid. This is a process allowing for cholinergic neuron returns to its resting state (Colovic et al., 2013)

As adult amphibians mainly feed on insects, they are greatly likely to be affected by insects contaminated by carbaryl. *Bufotes variabilis* is a species widely distributed throughout Turkey and is found in all suitable habitats (Başoğlu et al., 1994). Carbaryl is a widely used insecticide in Turkey (Delen et al., 2005). Therefore, this species is under a risk like other animals living in the areas where carbaryl is applied. However, nothing is known about the effects of carbaryl on this toad. While a large number of studies have been performed related to the effects of carbaryl on tadpoles (Bachetta et al., 2008; Distel and Boone, 2009; Kang et al., 2010; Boone et al., 2013), there is very little information on the effects of carbaryl in adult amphibians (Çakıcı, 2013, 2014). In addition, it is important to research the effects of pesticides in adult amphibians because they may have a potential to damage the essential organs. Liver is the crucial organ in the first pass of carbaryl metabolism (Munglang et al., 2009). In Amphibia, the kidney is an important organ in maintaining the water and salt balance and this balance is affected by xenobiotics which may cause disruptions in histologic achitecture of kidney (Paunescu et al., 2012). As a result of these damages, organisms can produce unhealthy offspring. Considering all, the aim of this study was to examine histopathological effects of carbaryl in liver and kidney tissues of *B. variabilis*.

2. Material and method

2.1. Ethics

The protocol was approved by the animal ethical committee of Ege University, Faculty of Medicine (2011-166).

* Tel.: +90 2323112420; fax: +90 2323881036.
E-mail address: ozlem.cakici@ege.edu.tr

http://dx.doi.org/10.1016/j.etp.2014.12.003
0940-2993/

2.2. Animal groups and experimental design

Adult individuals of *B. variabilis* were caught around İzmir/Turkey and maintained under laboratory conditions at a 12 h dark/light cycle, 22 ± 3° C temperature and 45 ± 5% relative humidity in the Herpetology Research Laboratory at Ege University Campus, Bornova-İzmir. After 15 days of acclimation, the toads were randomly assigned to either the carbaryl-treated groups or to the control group, each consisting of eight frogs (four females/four males).

As stated by Durant et al. (2007), contaminated insects are an important route of exposure to pesticides for insectivorous vertebrates inhabiting areas that receive pesticide application. Due to the fact that adult toads feed on insects, they may exposed to carbaryl by eating prey that is covered in carbaryl. But only one study made by Fair et al. (1995) directly quantified carbaryl residues of terrestrial invertebrates and they determined that grasshoppers had mean residues of 17 μg/g two days following rangeland application of 0.5 kg active ingredient/ha. In view of this insect residue data and carbaryl application rates, which can vary from 1.12 to 22.42 kg active ingredient/ha (EPA, 2004), Durant et al. (2007) reported that a 10 g lizard consuming 1 g prey could ingest dose concentrations ranging between 3.9 and 78.5 μg/g 2 days following carbaryl application. Factoring the short-half life into their estimates, authors determined three doses that entirely encompass the range of concentrations that they believed lizards could encounter in the environment. Based on these, carbaryl doses of this study were calculated. In the same way, it is believed that these three doses of carbaryl are the ones that toads could encounter in the environment.

Carbaryl was administrated once by oral gavage to experimental groups including low dose (0.05 mg/g), medium dose (0.1 mg/g) and high dose (0.2 mg/g). Carbaryl (purity 98%) was supplied by AgroBest Grup (İzmir, Turkey). It was dissolved in acetone. Prior to experiments, acetone control was made. 96 h following exposure to carbaryl, toads were euthanized and their organs were quickly removed.

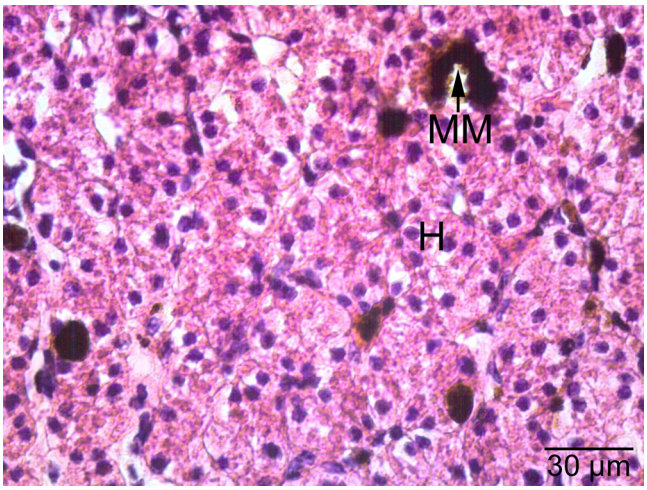

Fig. 1. Liver histologic section of control group, hepatocyte (H), melanomacrophages (MM).

2.3. Histological analyses

For light microscopic examination, liver and kidney tissues of the experimental toads were fixed in Bouin's fixative for 24 h, dehydrated in ethanol, cleared in xylol and embedded in paraffin. Serially sectioned tissues at 5 μm were stained with Mayer's Haematoxylin and Eosin and then photographed with Olympus CX31 (Tokyo/Japan).

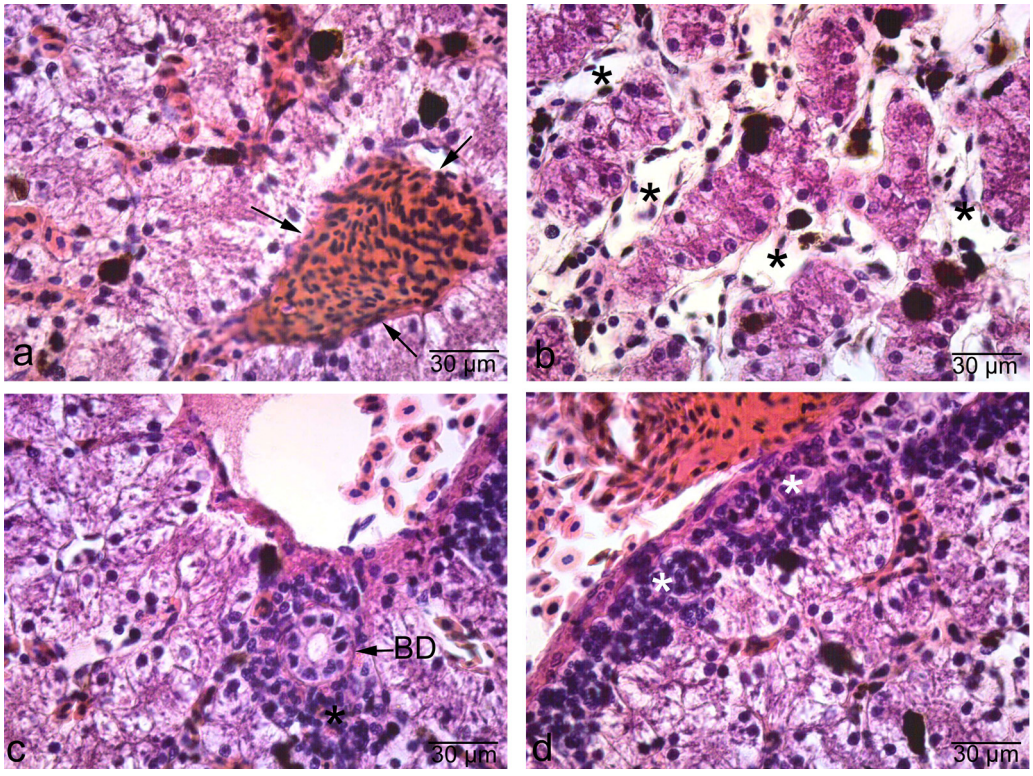

Fig. 2. (a) Liver histologic sections of low dose group, congestion (arrow). (b) Enlargement of sinusoids (asterisk). (c) Mononuclear cell infiltration (asterisk) seen around bile duct (BD). (d) Mononuclear cell infiltration (asterisk) seen around hepatocytes (arrow).